INDUCTION OF GENE POOL DIFFERENTIATION IN *DROSOPHILA MELANOGASTER*

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The selective effect of the ecological parameter "food medium" upon the gene pool of *Drosophila melanogaster* was investigated in nine cage populations by estimating the allozyme frequencies of the *a-Gpdh* and *Adh* loci. The differentiation observed was found to depend dramatically upon the action of the above mentioned environmental factor. Small differences may be mainly attributed to the effect of the differing genetic backgrounds of each gene pool. When the environment was restrictive, a dramatic gene pool differentiation was observed. A discussion is provided bearing upon the observed induction of a process which involves large groups of coadapted genes upon which selection actually acts. Furthermore, the role of nutrition, and especially yeast, was found to have a profound effect upon the observed genetic differentiation of the gene pool of *D. melanogaster*.

Neuf populations en cage ont été utilisées lors d'une étude sur les effets sélectifs du paramètre écologique "nourriture" sur le pool génique de *Drosophila melanogaster*. La fréquence des allozymes des locus *a-Gpdh* et *Adh* a été déterminée, et les différences que l'on a observées semblent fortement dépendre du facteur précité. Les légers écarts sont principalement attribués aux différences fondamentales des divers pools géniques qui présentent des distinctions très importantes en milieu restrictif. La discussion qui suit les résultats suppose l'induction d'un mécanisme impliquant d'importants groupes de gènes coadaptés sur lesquels se dirige l'action du processus de sélection. Par ailleurs, la nutrition et surtout la levure semblent agir profondément sur la différenciation génétique des pools de *D. melanogaster*. [Traduit par le journal]

Introduction

The study of the genetic structure of populations is one of the approaches to understanding the process of evolution. If one wishes simply to comprehend the evolutionary forces or to control them, it is helpful to evaluate the significance of the effect of certain ecological factors upon gene pool differentiation. Many studies have reported on temporal and geographical genic variations in natural populations associated with specific environmental conditions (for review see Parsons, 1973; Nei, 1975). However, data bearing upon these questions may be more appropriately obtained from laboratory cage populations where conditions may be readily controlled.

In view of the above, an attempt was made to observe the reaction of a large gene pool of *Drosophila melanogaster* to the ecological parameter "food medium" ("poor" food medium — restrictive environment; "rich" food medium — optimum environment). It is well known that yeast is the primary food source of *Drosophila* (Sang, 1949) and that yeast-culture media are very suitable for *Drosophila* cultures (Demerec, 1965). Thus, food medium poor in yeast generates a restrictive environment in which the flies live under limiting (competitive) conditions, while food medium rich in yeast defines an optimum (or near optimum) environment with no or little competition (Alahiotis, 1976). This investigation examines the idea that should strong competition for food and living space exist, natural selection will occur causing gene pool differentiation. In order to trace such a possible gene pool-environmental reaction, the allozyme frequencies of *a-Gpdh* (a-Glycerophosphate dehydrogenase) and *Adh* (alcohol dehydrogenase) were utilised. These two enzyme loci were found to associate nonrandomly with the polymorphic inversions of the second chromosome in the populations examined here (Alahiotis *et al.*, 1976). Hence, they can be considered as markers of large blocks of genes.

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Material and Methods

In our experiments, nine cage populations were studied for the allozyme frequency changes of two enzyme loci, over 30 generations, under the influence of the ecological factor food medium. Populations 1A, $1B_3$, $1B_4$, and 2A were raised at 25±0.5°C, 43±4% RH (relative humidity) in a poor corn meal-agar-sugar food medium (12.5 g corn meal, 1.8 g sugar, 1 g agar per 100 H_2O, with 1 ml of diluted living yeast suspension per food vial) to which propionic acid was added. Populations, 1B, $1B_1$, $1B_2$, 2B, $1A_1$ were exposed to the same conditions of temperature and relative humidity but raised in a rich dead-yeast-agar-sugar food medium (10 g dead-yeast, 10 g sugar, 3 g agar per 100 ml H_2O) where again propionic acid was added. Care was taken to have more or less the same amount of medium in all the vials used for the experiments. All these populations were maintained under a 12 h darklight cycle. During the early generations there was a rapid expansion of population size, which subsequently was regulated according to the kind of food medium on which each population was raised. Thus, the rich food medium populations had produced approximately the same number of flies (about 2,800) while, the poor-food medium population size was about 1,100 flies. (For more details see Alahiotis, 1976.)

Populations $1B_3$, $1B_4$ as well as $1B_1$, $1B_2$, were derived from the basic population 1B ten generations after the latter originated. The previously mentioned populations were put two-by-two ($1B_1$-$1B_2$, $1B_3$-$1B_4$) under the effect of the food medium (rich-poor food medium). Populations 1A, 1B originated from a common gene pool of 600 parents collected from a Cephalonian-Greek natural population, while populations 2A, 2B originated from a Gavros-Achaïa-Greek natural population. The above mentioned 600 parents originated from 100 isofemale lines derived from recently collected (summer 1973) individuals (populations were set up 20-30 days after the capture of flies). Six (3 virgin females and 3 males) 4-6 days old individuals were taken from each of the isofemale lines and thus the 300 virgin females and the 300 males gathered were allowed to mate randomly in the cage (1B or 2B). Populations 1A and 2A were derived by replicating the 1B or 2B populations respectively. Consequently, we may regard the derived populations of each series, as possessing practically the same gene pool. Moreover, in order to provide more evidence, a complementary experiment was carried out involving a new cage population (the $1A_1$) which was produced from the 16th generation of the 1A population.

The populations were maintained in plexiglass cages the dimensions of which were 41×41×16 cm, possessing 14 vials each (the dimensions of which were 10×2.5 cm). For details see Stern (1972).

In sampling the cages, three or four fresh food vials were placed in the cage. After 24 h the vials were removed and care was taken to avoid crowding in them. The vials were then placed under the same environmental conditions in which the respective populations were originally maintained, and emerging adults were scored for *a-Gpdh* (E.C.1.1.1.8.) and *Adh* loci (E.C.1.1.1.1.) genotypes. In order to characterize the enzyme genotypes of individual flies, starch gel electrophoresis was carried out at 300 volts, 100mA, for about one hour, using 0.5 M Tris versene-borate buffer pH 8.0 (Shaw and Prasad, 1970). Gells were sliced horizontally; the lower half was stained according to the method of Shaw and Prasad (1970) for *a-Gpdh*, while the upper half was stained for *Adh* using the method of Johnson and Denniston (1964).

Results

Our findings are presented in four tables and four figures. In all cage populations studied, two allozymes for each locus were found. Initial allozyme frequencies used were the frequencies found in the two aforementioned natural Southern Greek populations.

As shown in Table I, the allozyme frequencies of the *a-Gpdh* and *Adh* loci remain the same over 30 generations for the rich food medium populations (1B, $1B_1$, $1B_2$, 2B) with a slight difference as regards to the Adh^F frequency in population $1B_1$ which was contaminated with mites for some generations.

In contrast, a dramatic change was observed in the allozyme frequencies of the *a-Gpdh* and *Adh* loci for the poor medium populations over 30 generations (Table II). The fast allozyme of the *a-Gpdh* locus revealed an increase in its frequency for all

TABLE I

Frequency changes in *a-Gpdh*^{F} and *Adh*^{F} in 1B, $1B_1$, $1B_2$, 2B cage populations (rich food medium)

	Population 1B					
	a-Gpdh^F			*Adh*^{F}		
Generation	Frequency	Sample size*	SE**	Frequency	Sample size	SE
1	0.5458	240	0.032	0.8945	218	0.021
5	0.5957	188	0.036	0.9279	222	0.017
10	0.6231	260	0.030	0.9375	272	0.015
13	0.6392	316	0.027	0.8829	316	0.018
17	0.6154	182	0.036	0.8846	182	0.024
19	0.5753	186	0.036	0.9301	186	0.019
21	0.5833	120	0.045	0.9083	120	0.026
25	0.6000	230	0.032	0.8957	230	0.020
30	0.6067	178	0.037	0.9101	178	0.021
			Population $1B_1$			
10	0.6231	260	0.030	0.9375	272	0.015
11	0.5707	184	0.036	0.9185	184	0.020
13	0.6344	186	0.035	0.8656	186	0.025
16	0.5833	120	0.045	0.8583	120	0.032
23	0.6504	226	0.032	0.8142	226	0.026
			Population $1B_2$			
10	0.6231	260	0.030	0.9375	272	0.015
11	0.5833	192	0.036	0.9479	192	0.016
13	0.5941	170	0.038	0.8647	170	0.026
16	0.6846	130	0.041	0.8385	130	0.032
23	0.6696	224	0.031	0.8616	224	0.023
30	0.6897	174	0.035	0.9368	174	0.015
			Population 2B			
1	0.5663	196	0.035	0.9490	196	0.016
5	0.4531	192	0.036	0.9688	192	0.013
10	0.5562	160	0.039	0.9833	180	0.009
14	0.5000	232	0.033	0.9700	232	0.011
15	0.6373	102	0.048	0.9706	102	0.017
20	0.5246	122	0.045	0.9426	122	0.021
24	0.6842	224	0.031	0.8772	224	0.022
29	0.5741	182	0.039	0.8944	180	0.023

*The total number of chromosomes scored (see Tables I, II, III, IV).
**SE = Standard Error (see also Tables II, III).

populations studied while the *Adh* F_1 allozyme exhibited a decrease in its frequency for all populations studied. It seems that strong directional selection was operating throughout the duration of the experiment. In some cases (population 2A) an elimination of one allele occurs.

Figures 1-4 illustrate in a graphic way those of our findings which we considered to be of great interest. Dramatic changes are observed in allozyme frequencies when the populations are under competitive conditions (poor food medium population), whereas these frequencies remain stable under rich food conditions. In addition, good evidence for the effect of the "food medium" parameter upon allozyme frequency differentiation is demonstrated by cage population $1A_1$. In this case we observed (Table III; Figs 1, 2) a dramatic reversal, in the direction of allozyme frequencies for both loci when the population is treated first in the poor food medium (population 1A) and then in the rich one (population $1A_1$). After a few generations the new frequencies tend to approach

TABLE II

Frequency changes in *a-Gpdh*^{F} and *Adh*^{F} in 1A, 1B_3, 1B_4, 2A cage populations (poor food medium)

Generation	*a-Gpdh*^{F} Frequency	Sample size	SE	*Adh*^{F} Frequency	Sample size	SE
			Population 1A			
1	0.5458	240	0.032	0.8945	218	0.021
5	0.7674	172	0.032	0.5594	202	0.035
10	0.8275	342	0.020	0.4770	348	0.027
14	0.9062	416	0.014	0.2260	416	0.020
15	0.9470	556	0.012	0.2032	556	0.017
16	0.9200	118	0.025	0.1900	118	0.036
19	0.8817	186	0.024	0.2957	186	0.033
21	0.9043	94	0.030	0.3085	94	0.048
25	0.9558	226	0.014	0.5973	226	0.033
26	0.9900	150	0.008	0.5400	150	0.041
30	0.9943	174	0.006	0.5057	174	0.038
			Population 1B_3			
10	0.6231	260	0.030	0.9375	272	0.015
11	0.6548	168	0.037	0.8750	168	0.026
13	0.5885	192	0.036	0.8854	192	0.023
16	0.5914	186	0.036	0.9086	186	0.021
19	0.7448	192	0.031	0.9010	192	0.022
21	0.8193	238	0.025	0.5672	238	0.032
26	0.9152	224	0.019	0.5893	224	0.033
30	0.9444	180	0.017	0.5778	180	0.039
			Population 1B_4			
10	0.6231	260	0.030	0.9375	272	0.015
11	0.5667	180	0.037	0.8722	180	0.025
13	0.6139	202	0.034	0.8663	202	0.024
16	0.6827	208	0.032	0.6160	208	0.034
19	0.7474	190	0.032	0.7421	190	0.032
21	0.8500	260	0.022	0.5346	260	0.031
26	0.9444	162	0.018	0.6392	158	0.038
30	0.9891	184	0.008	0.5761	184	0.036
			Population 2A			
1	0.5663	196	0.035	0.9490	196	0.016
5	0.6310	168	0.037	0.7440	168	0.034
10	0.8059	304	0.023	0.6833	300	0.029
14	0.6200	660	0.019	0.7100	360	0.018
20	0.9022	92	0.031	0.5761	92	0.052
24	0.9439	214	0.016	0.6028	214	0.034
29	1.0000	138	—	0.5435	138	0.042

those of populations maintained in the rich food medium (1B, 1B_1, 1B_2, 2B). As far as the *Adh*^{f} frequency differentiation in the 1A population is concerned, it appears (Fig. 2) that there is a parallel increase of this frequency in both the 1A (poor medium) and 1A_1 (rich medium) populations, mainly between the period 20-25 generations. However, this is not true in later generations. Fig. 2 demonstrates an oscillation of the *Adh*^{F} frequency in the 1A population with a general decrease over the entire 30 generations resulting in a clear frequency differentiation between these populations. The difference in the *Adh*^{F} frequency between 1A and 1A_1 at the 30th generation is highly significant (x_1^2=9.6658 P<.01; according to Workman and Niswander 1970). It is possible that sampling errors or random drift may have been the contributing factors for the

oscillation observed (unexpectedly high frequency of Adh^F in 20-25 generations) in this population. Moreover, Table II shows clearly that, as a general rule, the Adh^F frequency decreases in every poor medium population throughout the 30 generations examined.

The results of testing for linkage equilibrium between the two loci (by comparing the observed with the expected combinations of the genotypes of *a-Gpdh* and *Adh*; Li, 1961) are given in Table IV, and show that these two loci are not generally in linkage equilibrium. Furthermore, in a recent publication (Alahiotis *et al.*, 1975) we have shown that nonrandom association exists between the allozymes of these loci and the

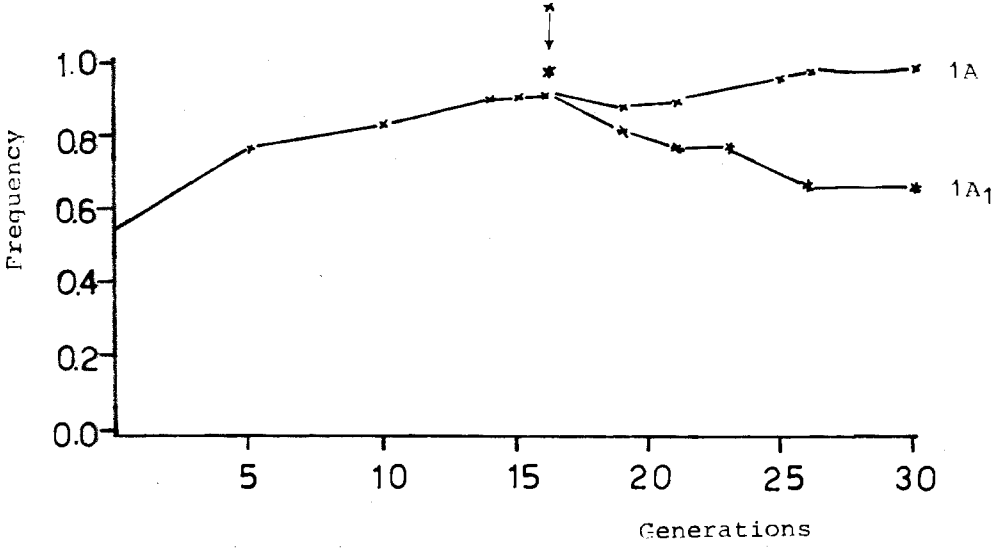

Fig. 1. Changes in frequency of *a-Gpdh*^{F} allele in 1A (x), $1A_1$ (*) cage populations (1A-poor food medium; $1A_1$-rich food medium).

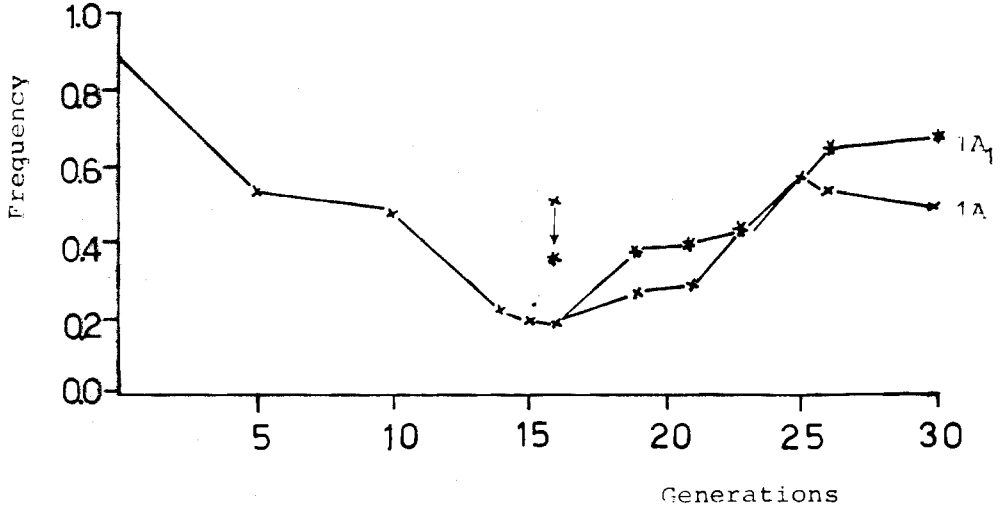

Fig. 2. Changes in frequency of Adh^F allele in the 1A (x), $1A_1$ (*) cage populations, (1A-poor food medium, $1A_1$-rich food medium).

TABLE III

Frequency changes in *a-Gpdh*^{F} and *Adh*^{F} in $1A_1$ cage population (rich food medium)

Population $1A_1$						
	Adh^F			*a-Gpdh*^{F}		
Generation	Frequency	Sample size	SE	Frequency	Sample size	SE
16	0.9200	118	0.025	0.1900	118	0.036
19	0.8253	166	0.029	0.3981	216	0.033
21	0.7793	290	0.024	0.4000	290	0.029
23	0.7902	224	0.027	0.4286	224	0.033
26	0.6699	206	0.033	0.6538	182	0.035
30	0.6786	196	0.033	0.6667	186	0.034

In(2L)22D-34A and *In(2R)52A-56F* inversions, both in cage populations as well as in a natural one. Thus, the existence of linkage disequilibrium between the allozymes of *Adh* and *a-Gpdh* loci may be considered the result of the presence of these inversions in the population studied. On the other hand, the fact that these two loci belong to two different metabolizing systems (Kojima *et al.*, 1970), decreases the probability of gene interaction between them.

Discussion

In the present investigation an attempt was made to induce gene pool differentiation under the influence of the environmental factor "poor-rich food

TABLE IV

A test for linkage equilibrium between the *a-Gpdh* and *Adh* loci in cage populations of *Drosophila melanogaster*

Populations (generations)		Probability (P)	Sample size	Populations (generations)		Probability (P)	Sample size
	13,5	<.001	316		14	<.001	416
	17	>.05	182		15	<.001	556
1B	19	>.05	186	1A	16	<.001	118
	21	>.05	120		19	<.001	186
	25	<.001	230		21	<.001	94
					25	<.001	226
	14	>.05	232				
	15	>.05	102		14	<.001	660
2B	20	>.05	122	2A	24	<.001	214
	24	>.05	228				
					4	<.001	192
	4	<.05	170		7	<.05	186
$1B_2$	7	>.05	130	$1B_3$	12	<.01	238
	14	<.01	224		17	<.01	224
	4	<.01	93		4	>.05	101
	7	<.001	60		7	<.01	104
$1B_1$	14	<.01	113	$1B_4$	12	<.01	130
	16	<.001	59				
	19	<.001	80				
$1A_1$	21	<.001	145				
	23	<.001	112				

medium". From our findings, it appears that the aforementioned differentiation is dramatic. The observed change in the allozyme frequency of a locus is in a specific direction for all populations studied under similar environmental conditions, thus suggesting the operation of directional selection. Furthermore, by utilizing the allozyme frequencies found in (Greek) natural populations as initial frequencies of the allozymes for the cage populations, we may assume that a given deviation from the initial (equilibrium) frequencies is due to the parameter under study.

Another factor worthy of consideration and capable of causing differentiation in the variability is genetic drift. In our experiments however, one could argue against genetic drift having played an important role on the basis of the following reasons: 1) large population sizes such that consecutive generations overlapped each other extensively, 2) the findings that changes in gene frequencies under the same environmental conditions were similar in many populations (small differences between populations with different original gene pool may be attributed to different genetic backgrounds), 3) the detection of a reverse frequency differentiation in the $1A_1$ population, 4) the existence of a proportional frequency differentiation in lethal bearing chromosomes (Alahiotis, 1976), and 5) in most of the cases our samples for each test were rather large. One cannot rule out the possible effect of genetic drift by the above arguments for the population sizes in poor food medium population were small ($N \simeq 1.100$). However, the maintainance of high lethal bearing chromosome frequencies (0.3900–0.4041: Alahiotis, 1976) in the same populations suggests the existence of a large Ne (effective populations size). The same is true, at least, in one of the natural populations (Yannopoulos, 1974) from which the founders were captured.

The density achieved by the cage populations studied depends on the type of food medium (poor food medium, high density-rich food medium, low density) used. Competition is one means by which selection can take place. Thus, competition in poor food medium populations must be high, while, it is low in the rich food medium populations. Here, one can only speculate that "mutual facilitation" may be of importance in polymorphic situations (like ours) where the different genotypes segregating in a population could have slightly different ecological requirements, thus resulting in a mutual facilitation (see Parson, 1973, for review). However, more data

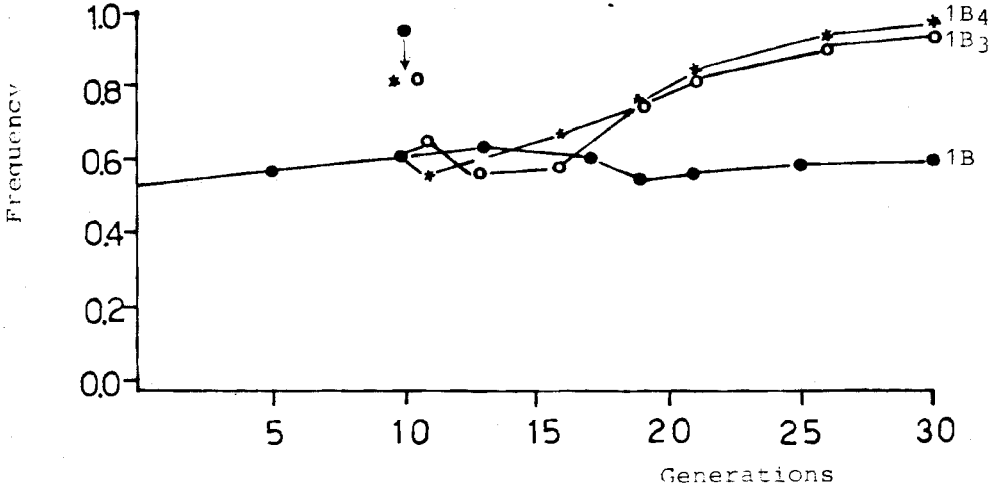

Fig. 3. Changes in frequency of *a-Gpdh*^{F} allele in the 1B (●), $1B_3$ (○), $1B_4$ (*) cage populations, ($1B_3$, $1B_4$-poor food medium; 1B-rich food medium).

(e.g. viability experiments) are needed so as to assess whether or not the above situation is true for our populations. It must also be noted that the small amount of live yeast suspension used in the poor medium is consumed by the flies in a few hours so that there is no live yeast left during larval development; hence, we can assume that competition exists for food (yeast) mainly in the larva stage. Thus, the observed dramatic gene pool differentiation could be attributed to the competitive conditions which reflect the difference of the food media. From this point of view it appears that nutrition, and especially yeast, may have some serious effect on gene pool differentiation and ultimately on the evolution of *Drosophila melanogaster*. It is important to note that we found strong sexual isolation (Isolation Index $\simeq 0.35$) in later generations among the same populations.

As already noted, the allozymes at both the *a-Gpdh* and *Adh* loci are nonrandomly associated with inversions on the second chromosome. The Adh^S and $a\text{-}Gpdh^F$ alleles are in strong linkage with the *In(2L)22D-34A*, thus suggesting that the frequency of the *In(2L)22D-34A* is much higher under competitive conditions than its' standard (see also Alahiotis *et al.*, 1976). These associations do not allow us to attribute the changes in allozyme frequencies to a selective effect at the loci studied. They may very well reflect a process which involves large groups of coadapted genes upon which selection acts (Prakash and Lewontin, 1968, 1971). The proportional frequency differentiation of the lethal bearing chromosomes (Alahiotis, 1976) as well as of the gene arrangements (Alahiotis *et al.*, 1975) and the linkage disequilibrium found between inversions of the second and third chromosomes (Alahiotis *et al.*, 1976) or between the alleles of the two loci studied, favors the above view.

However, in recent studies it has been suggested that allozymes do not constitute part of the coadapted complexes of genes characterizing inversions (Zouros, 1976). Moreover, linkage disequilibrium between allozymes and inversions is considered to be a historical accident where allozymes share their fate with the inversions (Watanabe and Watanabe, 1977). However, by studying additional cage populations (Alahiotis and Pelecanos, 1977) it has been shown that the *a-Gpdh* allele frequencies were found to be

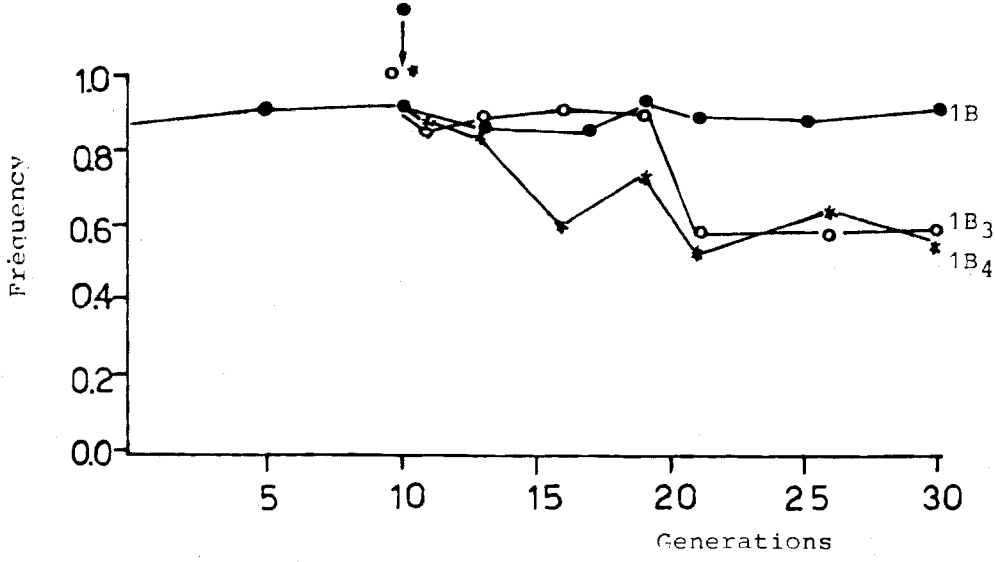

Fig. 4. Changes in frequency of Adh^F allele in the 1B (•), $1B_3$ (○), $1B_4$ (*) cage populations, ($1B_3$-$1B_4$-poor food medium; 1B-rich food medium).

uniform in relation to different second chromosome inversion frequencies $\{In(2L)22D\text{-}34A\}$, a fact which supports the view that while this locus is included in the inversions, it doesn't share its fate (e.g. in cage populations 1B, 1D, natural Greek population (f) $a\text{-}Gpdh^F$ = 0.6136, 0.6293, 0.6769 and (f) $In(2L)22D\text{-}34A$ = 0.0611, 0.4375, 0.0462 respectively). Furthermore, with respect ot the *Adh* locus, there is similar *Adh* allele frequency-temperature interaction in populations with low (Natural populations: Pipkin *et al.*, 1976) and high (our cage populations for the temperature effect: Alahiotis and Pelecanos, 1977) second chromosome *(2L)* inversion frequencies. Finally, since gene frequencies tend to be similar under the same environmental conditions in all cage populations studied, this favors the coadaptation hypothesis.

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